Increasing Water-Use Efficiency and Age-Specific Growth Responses of Old-Growth Ponderosa Pine Trees in the Northern Rockies

By: **Peter T. Soule´** & Paul A . Knapp

Abstract

We examined radial growth responses of ponderosa pine (Pinus ponderosa var. ponderosa) between 1905–1954 and 1955–2004 to determine if the effects of increased intrinsic water-use efficiencies (iWUE) caused by elevated atmospheric CO2 concentrations were age-specific. We collected 209 cores from five sites in the Northern Rockies and calculated iWUE using carbon isotope data from 1850 to 2004. Standardized radial growth responses were age dependent, with older trees exhibiting significantly higher values than younger trees during the later period at four sites and all sites combined. No significant differences in radial growth existed either for the individual sites or combined site during the earlier period. Increases in iWUE during 1955–2004 were 11% greater than during 1905–1954, and pentadal fluctuations in iWUE were significantly correlated with the radial growth of older trees from 1850 to 2004. Radial growth of younger trees and iWUE were not significantly correlated. Our results suggest that: (1) responses to elevated atmospheric CO2 in old-growth ponderosa forests are age-specific; (2) radial growth increases in older trees coincided with increased iWUE; (3) ponderosa had increased growth rates in their third, fourth, and fifth centuries of life; and (4) age-specific growth responses during 1955–2004 are unique since at least the mid-16th century.

Peter T. Soule´ & Paul A . Knapp (2010) "Increasing Water-Use Efficiency and Age-Specific Growth Responses of Old-Growth Ponderosa Pine Trees in the Northern Rockies" *Global Change Biology* Volume 17 pp. 631-641 Version of Record Available From (www.online.wiley.com)

Introduction

Atmospheric CO_2 concentrations have increased by over 27% since the early 20th century, resulting in enhanced radial tree growth in natural environments for numerous tree species in a variety of climatic regions (e.g., LaMarche *et al*., 1984; Knapp *et al*., 2001; Soulé & Knapp, 2006; Voelker *et al*., 2006; Wang *et al*., 2006; Koutavas, 2008). The principal benefit of elevated CO_2 for radial growth has been linked to increased intrinsic water-use efficiency (iWUE), which is the ratio of net CO_2 assimilation through leaf stomata to leaf stomatal conductance. Increases in iWUE based on carbon isotope chronologies have been identified for trees growing in both controlled (e.g., Leavitt *et al*., 2003) and natural environments (e.g., Bert *et al*., 1997; Feng, 1999; Tang *et al*., 1999; Arneth *et al*., 2002; Saurer *et al*., 2004; Waterhouse *et al*., 2004; Liu *et al*., 2007). However, not all have found this relationship to be universal (e.g., Marshall & Monserud, 1996) and sensitivity to increased iWUE may decline over time for some species (Waterhouse *et al*., 2004).

Feng (1999) has posited that naturally growing trees, particularly those growing under drier climate regimes, would experience enhanced radial growth rates in response to CO_2 enrichment. Because soil moisture is often the limiting factor to radial growth in semiarid and arid environments, the influence of elevated atmospheric CO_2 may be most pronounced in these environments (Huang *et al*., 2007). The concept of maximum response in drier environments is supported by the results of controlled studies, which suggest that water stress tends to enhance the *relative* effects of elevated CO_2 on woody plant growth (Idso & Idso, 1994; Poorter & Perez-Soba, 2001; Wullschleger *et al*., 2002). One of the primary effects of elevated CO_2 is a reduction in the stomatal openings of tree leaves during photosynthesis (e.g., Tognetti *et al*., 1998), which reduces transpiration rates and results in increased iWUE. Thus, radial tree growth may continue later through the summer in environments where soil moisture becomes limiting by July or August (e.g., the Pacific Northwest and Northern Rockies), or growth reductions may be ameliorated during a drought when the benefits of increased WUE are typically most pronounced (Wullschleger *et al*., 2002).

Age-related declines in growth after canopy closure have been associated with higher respiration rates,

nutrient limitations, and genetic changes (see overview by Ryan & Yoder, 1997), as well as hydraulic limitations (Ryan *et al*., 2006) and reductions in photosynthesis (Yoder *et al*., 1994). However, recent work regarding the importance of aging temperate forests and woodlands as carbon sinks (e.g., Carey *et al*., 2001; Zhou *et al*., 2006; Luo *et al*., 2007; Luyssaert *et al*., 2009) suggest that these factors may not be universally operative. Old-growth ponderosa pine trees can maintain or increase productivity with age (e.g., Kaufmann, 1996), particularly when competition is reduced (e.g., McDowell *et al*., 2003). In the Northern Rockies, Carey *et al*. (2001) found that annual net primary productivity increased with age in subalpine forests because respiration rates decreased with tree age, and Law *et al*. (2001) concluded that net primary productivity of old-growth ponderosa pine (*Pinus ponderosa* var. *ponderosa*) in central Oregon was higher than for a younger cohort.

Tree-ring growth responses to climate may be dependent on tree age, often with older trees being more sensitive (e.g., Carrer & Urbinati, 2004; Yu *et al*., 2008; Wang *et al*., 2009) possibly because changes in hydraulic status may make trees more susceptible to stress (Carrer & Urbinati, 2004). Along analogous lines, a less discussed, but intriguing question has been posited by Phillips *et al*. (2008) regarding the potential capacity of increased growth in older trees because of increased atmospheric CO_2. This idea parallels questions regarding the potential effects of anthropogenically caused climate change on plant communities, which have been directly addressed for pine forests by Richardson *et al*. (2007) and by Williams & Jackson (2007, p. 475), who discussed the implications of future 'novel climates' where no historical precedent exists of how ecological communities will respond. Similarly, if CO_2 fertilization is an operative process in many natural forests, then this age-related physiological process may be novel to the Holocene as well. We posit the question: Are the benefits of increased growth related to changing environmental conditions age-specific in trees given that some species have age-dependent responses to climate stress? In this paper, we use tree-ring data collected from ponderosa pine trees at five semiarid sites in the Northern Rockies to examine if: (1) the response to rising atmospheric CO_2 is age-sensitive; (2) increases in radial growth correspond with increased iWUE; and (3) the observed changes are unique to at least the mid-16th century.

Materials and methods

Tree-ring sampling and chronology development

We developed tree-ring chronologies from data collected at four locations in western Montana (BCR, FCP, FLR, RCP), and one location (SRC) in eastern Idaho (Table 1, Fig. 1). We selected sites that had minimal anthropogenic disturbances (i.e., fire suppression, livestock grazing, and logging) and were open woodlands comprised of either ponderosa pine or ponderosa pine mixed with Douglas-fir (*Pseudotsuga menziesii* var. *glauca*). All sites were comprised of all age-categories of trees located on south-facing slopes to ensure greater soil moisture stress (Fig. 2). At each site we selectively sampled approximately 40–50 visually healthy trees from multiple age classes. We avoided trees with major fire scars, spiked tops, heavy mistletoe infections (Stanton, 2007), canopy infringement, aggregation with other trees, or any other known growth-altering characteristics. We collected two core samples per tree at approximately 1.4 m height using standard field techniques (Phipps, 1985). For each tree sample, we measured basal diameter and height and recorded information on any micro-environmental factors that may have impacted tree growth. Our sampling was designed to be heterogeneous and representative of whole-site conditions. Thus, if anything about an individual tree or a portion of our study site seemed abnormal we avoided it, and the mix of old and young trees we selected was as spatially random as possible.

We sanded, crossdated, and measured cores for each site following standard dendrochronological procedures (Stokes & Smiley, 1968; Yamaguchi, 1991). We used the program COFECHA (Holmes, 1983) to confirm crossdating and measurement

Table 1 General information about the five study sites and chronology statistics

Site name	# Dated series	Master series	Series inter-correlation	Mean sensitivity	Coordinates (°)	Elevation (m)	Mean height and range (m)	Mean estimated interior date (years)	RBAR and (EPS) of standardized chronologies
BCR	24	1560–2004	0.598	0.248	45.83, −114.25	1555	34.2 (22.8–47.0)	1725	0.452 (0.952)
FCP	48	1575–2006	0.576	0.252	46.86, −114.69	1209	26.9 (11.5–54.9)	1820	0.344 (0.959)
FLR	54	1560–2004	0.638	0.265	47.33, −114.87	1214	29.7 (19.4–58.0)	1755	0.426 (0.975)
RCP	47	1602–2006	0.655	0.295	46.95, −114.33	1683	31.5 (20.0–58.1)	1768	0.472 (0.975)
SRC	36	1690–2004	0.695	0.347	45.33, −114.44	1230	27.8 (23.9–39.3)	1817	0.578 (0.977)

RBAR values measure the mean correlation between the individual tree-ring series during the common overlap period (i.e., common variance; Cook & Briffa, 1990), whereas EPS is an indicator of chronology reliability with values >0.85 considered acceptable (Wigley *et al*., 1984).

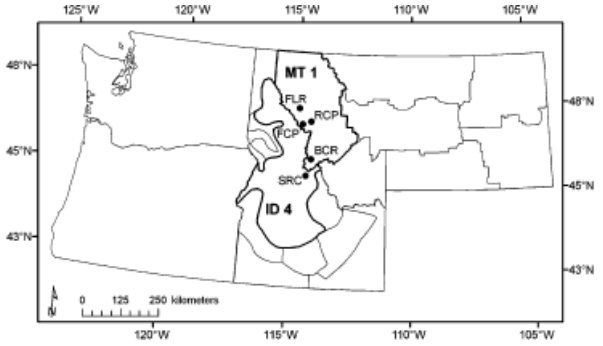

Fig. 1 Location of study sites and climatic division boundaries in Montana and Idaho. The two climate divisions selected are highlighted.

Fig. 2 Open ponderosa pine woodlands at the SRC site.

accuracy for each chronology. We determined the interior age for each core using a pith estimator providing that ring curvature was present, and cores without curvature were excluded from further analysis. Additionally, because we wished to analyze trees that reached maturity before elevated CO_2 levels, we omitted any tree without a measurable tree ring before 1905. For each tree, we used the oldest estimated date among samples as the interior date at 1.4 m height. The final data set consisted entirely of mature trees ranging in age from at least 100 to over 450 years.

We standardized samples that met the above criteria using negative exponential, negative regression, or a horizontal line through the mean using the program ARSTAN (Cook, 1985). In our analyses, we used individual tree standardizations, developed from each chronology, to determine age-specific growth responses and site chronologies for growth/climate modeling. In instances where indices were averaged either by site or all sites combined, we again used data from the individual chronologies. All the indices were developed from the STNRD version of ARSTAN because we wished to preserve low-frequency variance preferable for analyzing multidecadal trends in our data while the use of a RESID chronology would remove this variance (Grissino-Mayer, 1996).

We checked for potential bias in the tree-ring indices that could lead to an artificial inflation of the data at the end of the record (Cook & Peters, 1997) by examining the mean ring width of the individual cores from 1955 to 2004. Although a small percentage of cores (i.e., 4.3%, Fig. 3) had a mean growth <0.5 mm during this period and thus fell into the potential 'danger zone' identified by Cook & Peters (1997, p. 364), we determined that their individual standardization curves did not markedly diverge either positively or negatively from the ring-width measurements during the past 50 years. As these cores otherwise met all our criteria for selection, we retained them for analysis in the interest of maximizing sample size. We additionally examined raw ring-width measurements during our study period and divided mean values for 1955–2004 by mean values for 1905–1954 to derive a ratio of radial growth to compare with our standardized indices.

Tree-ring analyses

We compared radial growth during 1905–1954 (near preindustrial CO_2, mean = 304 ppmv) and 1955–2004 (elevated CO_2, mean = 340 ppmv) for each site chronology and all sites combined. To compare these time periods, we used bivariate linear regression with radial growth and interior age as the dependent and independent variables, respectively, and then determined the significance of the trend line for each model. Additionally, we compared mean radial growth values between time periods for each site and all sites combined using the Mann–Whitney test (Hollander & Wolfe, 1999). To determine if differences in radial growth rates between the 1905–1954 and 1955–2004 periods were associated with either tree height or diameter, we calculated differences in radial growth between the early and late periods for each core and then used this value in a regression model with the associated height or diameter as the dependent variable.

Isotopic analyses

We developed carbon isotope chronologies of ponderosa pine to measure carbon isotope composition ($\delta^{13}C$) of the wood cellulose from 1850 to 2004 at BCR, FLR, RCP, and SRC (FCP was collected at a later date and thus was not available for isotopic analysis). We selected six trees per study site with each tree represented by two quality cores with clear ring structure. Where possible, each core had pre-1800 interior dates with pith present or estimatable from the interior ring structure. Because the innermost 25 rings from the pith need to be excluded from the analysis to avoid isotope 'juvenile effects' (Freyer, 1979; McCarroll & Loader, 2004, p. 789), we conducted our isotopic analyses using the time period 1850–2004.

Caution is necessary when interpreting iWUE values as Seibt *et al*. (2008, p. 451) note that 'δ^{13}_{plant} trends alone are not reliable indicators of changes in plant water use efficiency without independent estimates of gas exchange or environmental conditions.' Accordingly, we present a detailed analysis of the climatic factors that influence radial growth and show that none of these variables significantly changed at four of our five study sites. For example, there is no evidence that 'shifts in atmospheric circulation . . . affect[ing] the evaporative

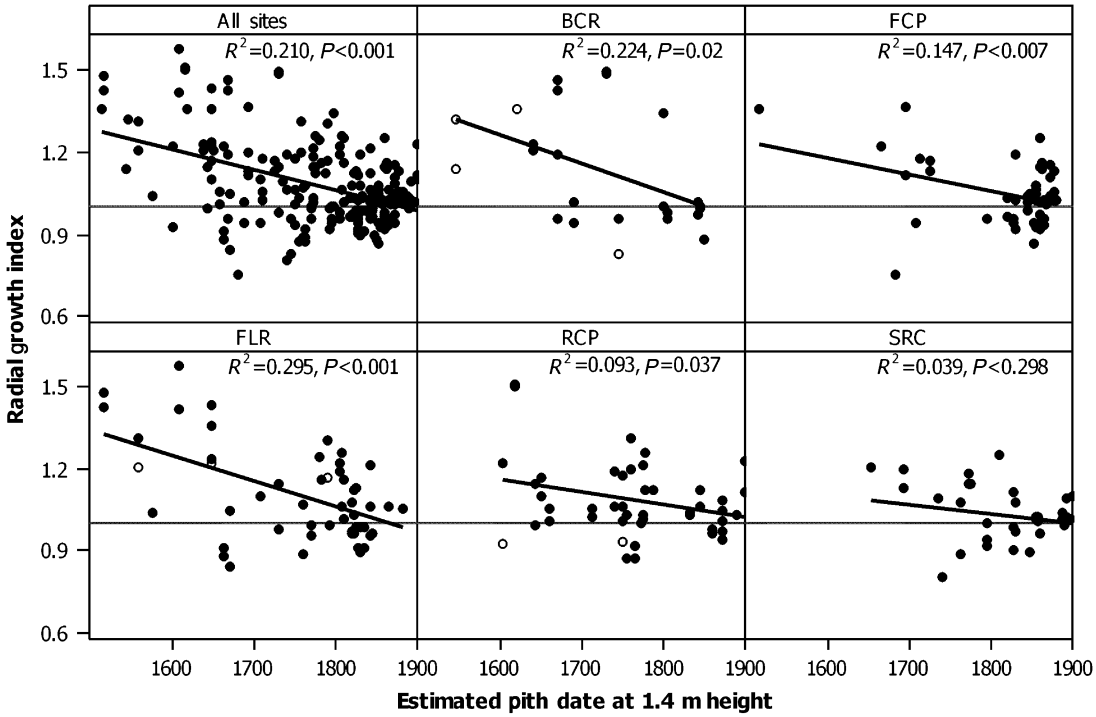

Fig. 3 Mean standardized radial growth during 1955–2004 as a function of tree age. The sloping line is best-fit linear regression. R^2 and significance values from linear regression models using radial growth (dependent) and tree age at selected time intervals for the five study sites and all sites combined are listed in the upper right corner. Core samples with mean raw ring-widths <0.5 mm ($n = 9$) are marked by open circles for individual sites.

demand' (Seibt *et al*., 2008, p. 451) of the trees have occurred in our study area. Additionally, our sampling process was designed to control/minimize as many other environmental factors as possible. Thus, in the context the data are presented, iWUE remains an effective means to examine centennial-length changes in water use.

We manually separated tree rings into 5-year segments (pentads) ending in 2000–2004 using a scalpel. We then pooled 5-year ring groups together from all trees for most pentads and ground them together to 40 mesh. Every 50-year period the trees were pooled and ground separately to quantify intertree isotopic variability. We processed all ground samples to α-cellulose after initial conversion to holocellulose with the Jayme–Wise method as implemented by Leavitt & Danzer (1993) using batch processing and commercial digestion pouches (ANKOM Technology, Boston, MA, USA). We first removed extractions from the samples using a soxhlet extraction apparatus operating successively with toluene/ethanol and ethanol organic solvents, and then by boiling in deionized (DI) water. We removed lignin by reaction in an acetic acid-acidified, sodium chlorite aqueous solution at 70 °C, followed by thorough rinsing in DI water. We accomplished the final isolation of α-cellulose by treatment in a 17% NaOH solution according to methodology described in Sternberg (1989) whereby samples were rinsed in DI water, and then dried at 70 °C.

We combusted the α-cellulose samples to CO_2 in a Thermo Finnigan TC/EA, which was delivered to a Thermo Finnigan Delta Plus XL mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) operating in flow-through mode via a Conflo III interface. We computed the isotopic composition ($\delta^{13}C$) with respect to the PDB standard (Coplen, 1996). We ran a homogenous acetanilide working standard of known isotopic composition approximately every four to five samples to calibrate the mass spectrometer, indicating machine precision of ca. 0.02–0.11‰ (SD) for each batch of 30–40 samples. We also used a holocellulose laboratory standard (MAWS) approximately every 15 samples, which established precision of 0.12‰.

Determining iWUE

We determined iWUE by using the equation (Ehleringer & Cerling, 1995)

$$\text{iWUE} = A/g = (c_i/c_a)0.625, \quad (1)$$

where A is the CO_2 assimilation by the ponderosa pine leaves, g the leaf stomatal conductance, 0.625 the constant, and c_i and c_a are the intercellular CO_2 and atmospheric CO_2. We interpolated annual c_a using decadal observations beginning in 1800 (Etheridge *et al*., 1998) and from Mauna Loa measurements from 1959 to 2004 (Keeling *et al*., 2008). c_i values were derived through Eqns (2) and (3) (Farquhar *et al*., 1982) for carbon isotopic discrimination for plants Δ (2) and the c_i/c_a ratio (3):

$$\Delta = (\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p/1000), \quad (2)$$

$$c_i/c_a = (\Delta - a)/(b - a). \quad (3)$$

We obtained the stable carbon isotopic composition of the atmosphere ($\delta^{13}C_a$) using data from Francey *et al*. (1999) and Allison *et al*. (2003) from 1796 to 2002. Annual data were temporally incomplete until 1991 and thus we used a sixth-order polynomial to fit the data to annual resolution (e.g., Hemming *et al*., 1998). We averaged $\delta^{13}C_a$ values by pentad and with all pre-1850 data held stable at −6.4‰. $\delta^{13}C_p$ represents the isotopic composition of the trees that were pooled by site and again averaged by pentad, and a and b represent constants for the discrimination during diffusion of CO_2 into the air (4.4‰) and discrimination during carboxylation (assumed at 27‰), respectively.

To determine if increased iWUE is associated with age-specific radial growth responses, we regressed pentadal iWUE values with pentadal values of radial growth using two sets of cores during 1850–2004. The first set was comprised of older trees with all cores having interior dates preceding 1750 (hereafter, OT, $n = 64$ cores). The second set was comprised of younger trees with interiors dates of 1850–1900 (hereafter, YT, $n = 56$ cores). Cores with interior dates from 1750 to 1849 ($n = 89$ cores) were treated as a transition period (i.e., without a gap, it would be possible to place trees with interior dates only 1 year apart into separate categories) and omitted from analyses. The OT and YT data sets were also regressed against pentadal CO_2 values during the same period to determine the relationship with radial growth.

Climate analyses

For each of the five sites, we developed multiple regression models predicting standardized radial growth as a function of climatic conditions. We used the 100-year time period 1905–2004 and obtained measurements of monthly mean temperature, total precipitation, and the Palmer Drought Severity Index (Palmer, 1965) for Montana Climatic Division One (BCR, FCP, FLR, RCP) and Idaho Climatic Division Four (SRC) from NOAA (NCDC, 2008, Fig 1). We initially examined relationships using the monthly PDSI, precipitation and temperature data, 1-year lagged values of the monthly data, season averages (e.g., December–February averages for winter), annual averages based on multiple 12-month periods (i.e., previous year September to current year August; previous year October to current year September), and various multimonth averages (e.g., January–March; February–April). We used both Pearson correlation and stepwise regression to identify potential models and developed the final models for each site based on logic, predictive ability, and statistical significance of the explanatory variables. We used the Variance Inflation Factor to check for multicollinearity among the explanatory variables.

To determine if there had been any significant changes in climatic conditions, we examined the linear trends of all monthly variables, the calendar annual means, and seasonal means using the 100-year period 1905–2004. We also tested for significant differences in these same variables, and all variables included in the growth/climate models, between the periods 1904–1954 and 1955–2004 using the Mann–Whitney test.

Results

Significant differences in radial growth responses based on age occurred at four of the five sites (BCR, FCP, FLR and RCP) and all sites combined (COMBO), with older trees exhibiting higher growth indices than younger trees during 1955–2004 (Fig. 3). Conversely, neither the individual sites nor COMBO had age-dependent growth responses either during 1905–1954 (Fig. 4) or any other sequential 50-year period (COMBO) dating to the mid-16th century (Fig. 5). Trees with interior dates from the 16th (mean growth index = 1.29) and 17th (mean growth index = 1.17) centuries had significantly ($P < 0.01$) greater growth indices than trees with interior dates from the 19th century (mean growth index = 1.03). However, this relationship was not significant ($P > 0.05$) for COMBO based on 1905–1954 values, as older trees (16th and 17th century mean growth indices = 1.01 and 0.96, respectively) and younger trees (19th century mean = 0.99) had similar growth indices. Differences in radial growth between the early and late periods were not significantly associated ($P > 0.05$) with either tree height or diameter at four of the five sites and COMBO. At BCR there were significant, but negative, associations with both height and diameter ($r = -0.417$, $P = 0.043$; $r = -0.454$, $P = 0.026$, respectively). Further, differences between the early and late periods are unlikely an artifact of standardization as similar and significant age-growth responses were derived using raw ring-width data (Fig. 6).

The growth/climate models suggest a high degree of similarity in radial growth rate responses among the five study sites (Table 2). All of the models contain variables that reflect a positive response to moisture conditions in both the current growing season (e.g., total precipitation from May through July and July PDSI values) and the latter part of the previous year's growing season (e.g., drought severity measured in September or October of the previous year). All of the variables included in the growth/climate models have no significant linear trends ($P > 0.05$) and have no significant differences when comparing the early (1905–1954) and late (1955–2004) periods.

Trends in monthly, seasonal, and calendar year climatic variables show that climatic conditions in the region have either not changed, or are trending in a direction that should not result in significant increases in radial growth. For Montana Climate Division 1 (all sites except SRC), the only variable with a significant ($P < 0.05$) long-term trend is calendar-year annual temperature. While this trend is positive, none of the sites displayed any significant relationships between annual temperature and radial growth. In Idaho Climate Division 4, we found significant negative trends in PDSI values for several months, annually, and during the spring. We also found that annual, winter, spring, and March temperatures are positively trending. While the two measures of drought severity most strongly related to radial growth at

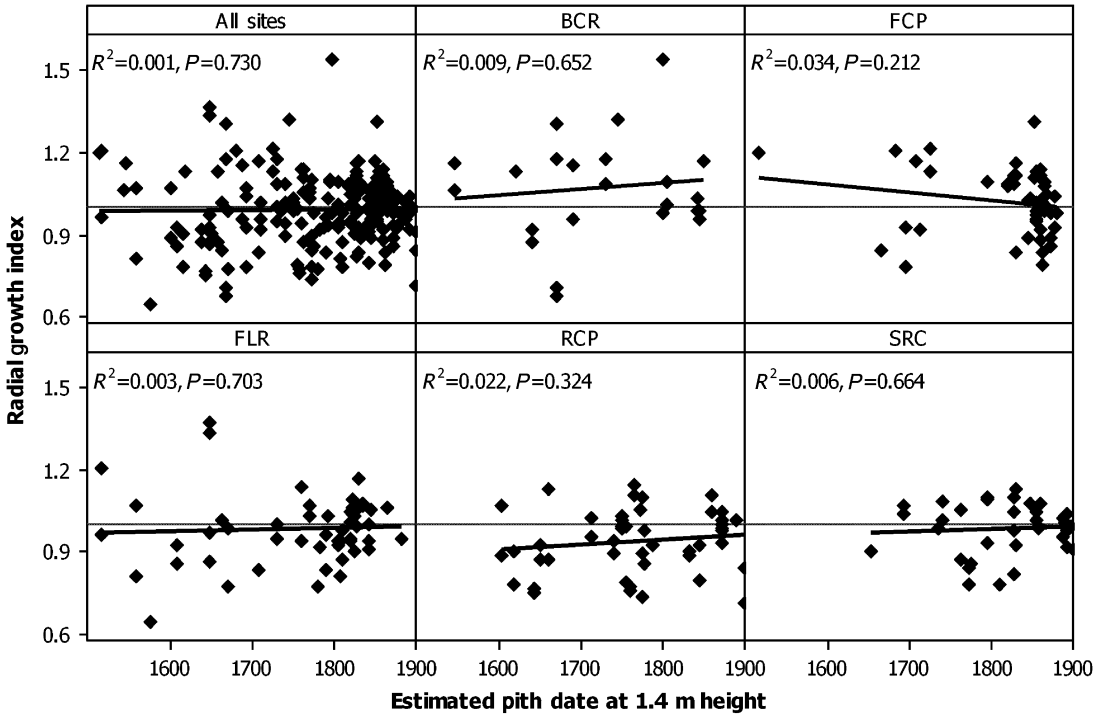

Fig. 4 Mean standardized radial growth during 1905–1954 as a function of tree age. The sloping line is best-fit linear regression. R^2 and significance values from linear regression models using radial growth (dependent) and tree age at selected time intervals for the five study sites and all sites combined are listed in the upper left corner.

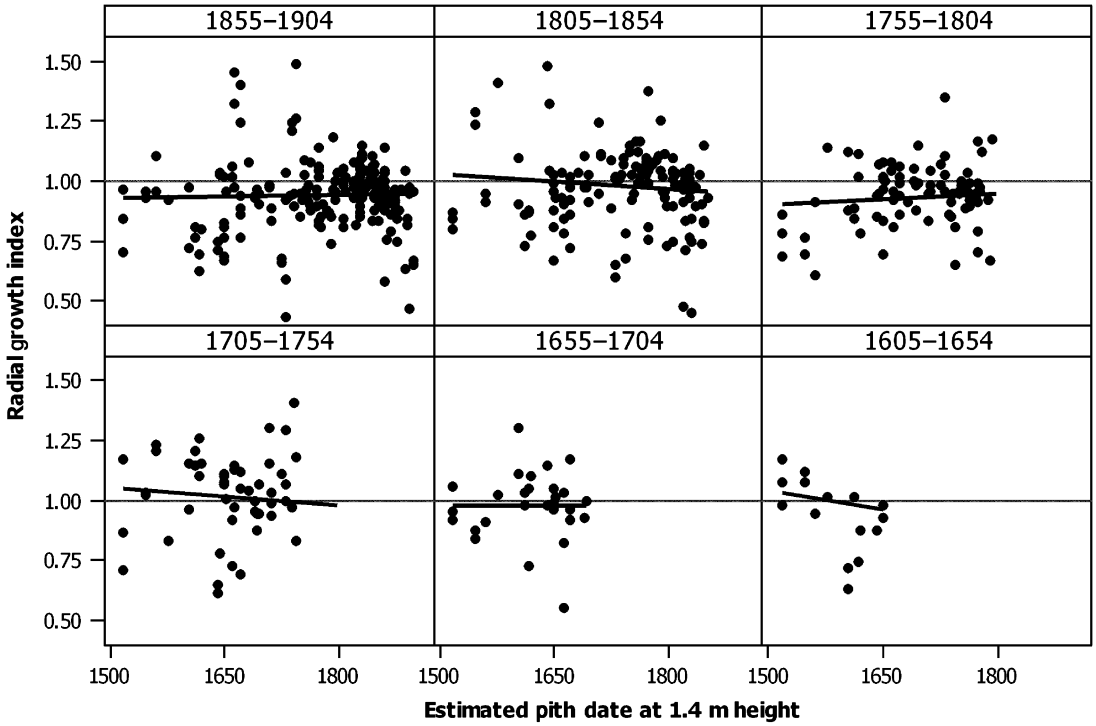

Fig. 5 Radial growth responses as a function of tree age for all sites combined for sequential 50-year periods. The solid sloping line is best-fit linear regression and no slope was significant ($P > 0.05$) for any time period including the earliest complete measured 50-year period of 1555–1604 (not shown).

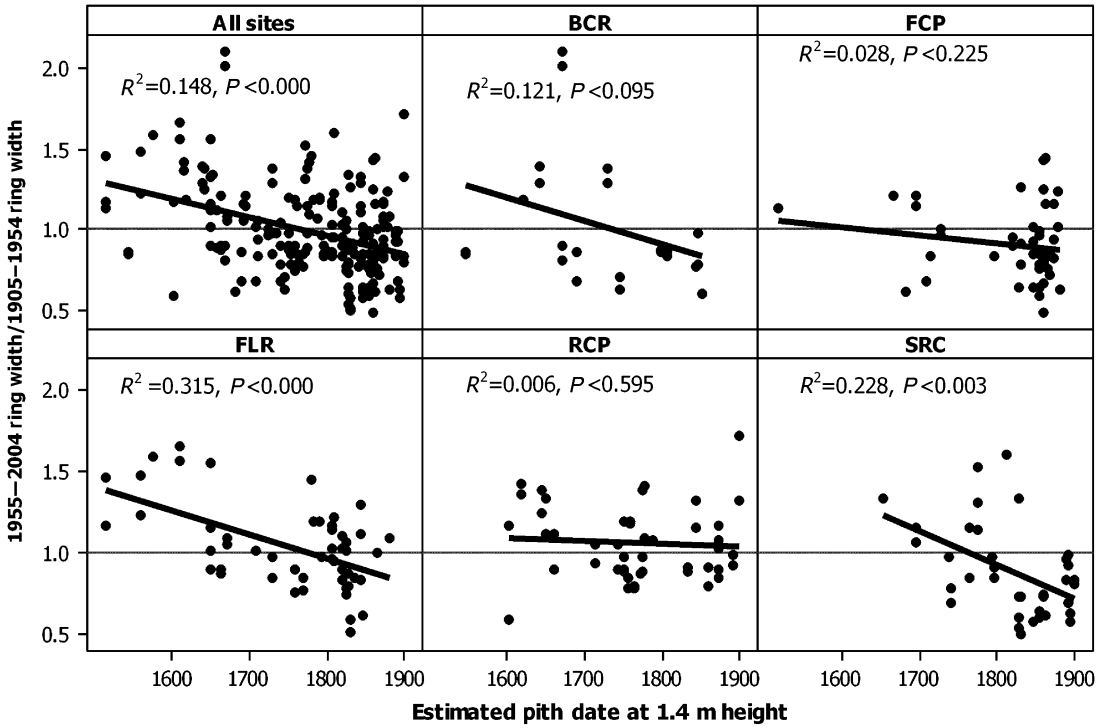

Fig. 6 Mean radial growth from the late period divided by the early period based on raw ring widths and shown as a function of tree age. The sloping line is best-fit linear regression. R^2 and significance values from linear regression models using radial growth ratios (dependent) and tree age at selected time intervals for the five study sites and all sites combined are listed above.

Table 2 Statistics from growth–climate models

	Growth/climate					
Site	Model variables*	Standardized β†	Partial R^2‡	Linear trend§	Linear trend *P*-value	Early/late comparison¶ *P*-value
BCR	mjjppt	0.462	0.305	−0.009	0.927	0.436
	l1seppd	0.366	0.124	−0.024	0.809	0.287
	aprtemp	0.198	0.039	0.038	0.706	0.815
FCP	l1octpd	0.417	0.236	−0.05	0.618	0.454
	mjjppt	0.383	0.143	−0.009	0.927	0.436
	maytemp	−0.204	0.036	0.123	0.222	0.533
FLR	julpd	0.387	0.273	−0.072	0.478	0.617
	l1octpd	0.287	0.069	−0.05	0.618	0.454
RCP	mjjppt	0.491	0.303	−0.009	0.927	0.436
	l1seppd	0.322	0.1	−0.024	0.809	0.287
SRC	l1octpd	0.449	0.321	−0.168	0.095	0.647
	seppd	0.288	0.069	−0.11	0.277	0.684

*mjjppt, total precipitation from May, June, and July of current year (cm); l1seppd, PDSI value from September of the previous year; aprtemp, average temperature in April of the current year (°C); l1octpd, PDSI value from October of the previous year; maytemp, average temperature in May of the current year (°C); julpd, PDSI value from July of the current year; seppd, PDSI value from September of the current year.

†Standardized regression coefficient for the growth/climate model variable.

‡Partial R^2-value for the growth/climate model variable.

§Pearson *r*-value between the growth/climate model variable and time, 1905–2004.

¶*P*-value from a Mann–Whitney test comparing values of the growth/climate model variable between the early (1905–1954) and late (1955–2004) periods.

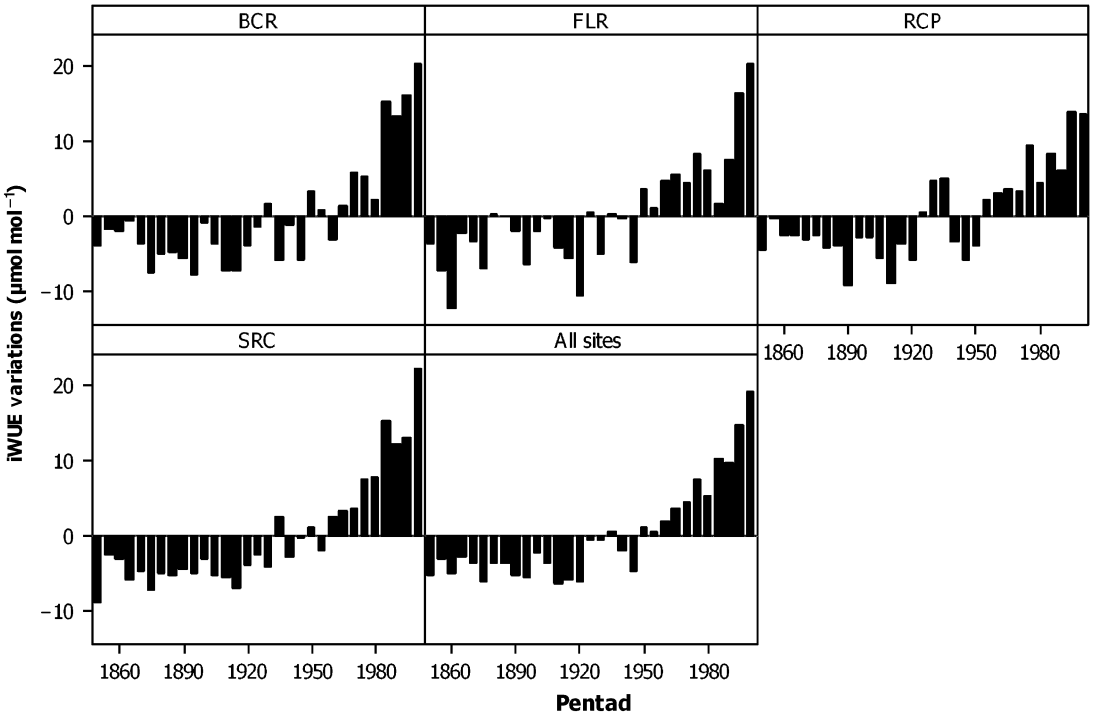

Fig. 7 Changes in intrinsic water-use efficiencies (iWUE) for ponderosa pine samples collected at four sites and all sites combined. Bars represent absolute changes in iWUE (5-year averages) based on average iWUE from 1850 to 2004. Trends for each site and the combined site increased significantly ($P < 0.01$).

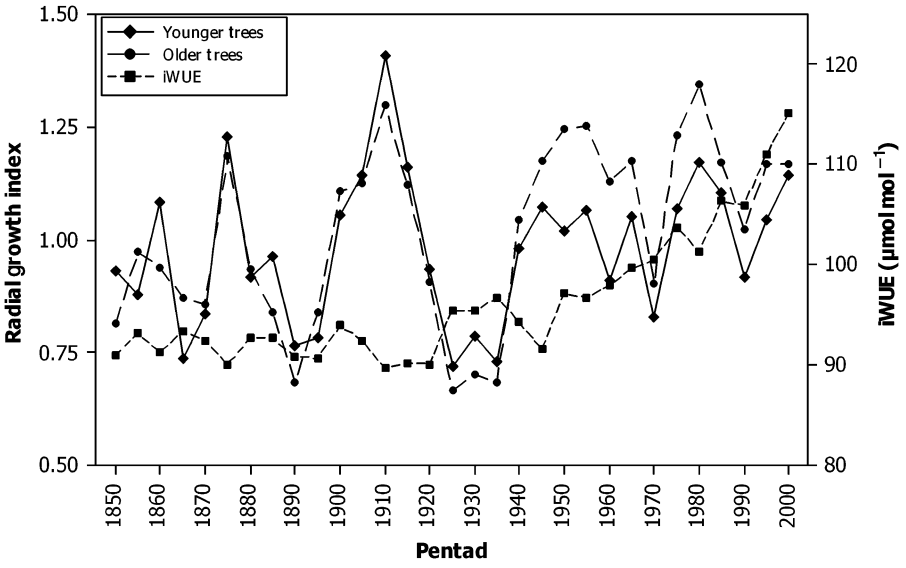

Fig. 8 Pentadal variability in radial growth from 1850 through 2004 for trees with interior dates preceding 1750 [older trees (OT), circles] and with interior dates no earlier than 1850 [younger trees (YT), diamonds]. Intrinsic water-use efficiencies (iWUE) (squares) and OT radial growth were significantly associated ($R^2 = 0.13$, $P < 0.05$), whereas YT and iWUE were not ($R^2 = 0.01$, $P > 0.05$).

SRC have no significant long-term trends or difference between the EARLY and LATE periods (Table 2), radial growth is positively related to the PDSI so the overall trends toward increasing aridity would not be conducive for enhanced growth of ponderosa pine.

iWUE increased significantly ($P < 0.01$) at the five individual sites and COMBO from 1850 to 2004 (Fig. 7). With the exception of the 1935–1939 pentad (associated with a corresponding dry period), values remained below the 155-year average until the 1950–1954 pentad.

Thereafter, values increased dramatically and remained above average. Variations among sites were minor, with three of the four sites experiencing the highest iWUE values of the entire record during the 2000–2004 pentad. Average iWUE values during 1955–2004 ranged from 11% to 12% higher than 1905–1954 at the individual sites and 11% higher for all sites combined.

iWUE was significantly related to radial growth of OT during 1850–2004 ($r = 0.339$, $P = 0.031$, one-tailed), but not with YT ($r = 0.113$, $P = 0.273$, one-tailed, Fig. 8). The mean radial growth index value for OT (1.16) was significantly greater ($P < 0.001$) than for YT (1.03) during 1955–2004. Conversely, before 1955–2004 (i.e., 1850–1954) no significant difference ($P = 0.946$) between OT (radial growth index = 0.95) and YT (0.96) existed. Trends in radial growth during 1850–2004 were significant and positive for OT ($R^2 = 0.225$, $P = 0.007$), but not significant for YT ($R^2 = 0.047$, $P = 0.242$). OT radial growth for the 1850–2004 pentads was significantly related to CO_2 ($r = 0.433$, $P = 0.015$), but not for YT ($r = 0.232$, $P = 0.208$).

Discussion

Age-specific radial growth responses of ponderosa pine during 1955–2004 are unique to the past 450 years (Fig. 5). These results suggest that some type of environmental change, such as increased iWUE in the latter 20th century (Fig. 7), has preferentially favored older trees, helping them grow at above-average rates while younger trees did not. Further, as this growth pattern occurred at four of the five study sites, it suggests that the cause(s) for the change are independent of site variability and at least regional in scale. At SRC, the lack of a significant age–growth relationship was likely because of limited sample size of older trees (Fig. 3), as the same pattern of increased growth based on age existed.

Similarities in growth responses suggest a regional-scale environmental driver, but it is unlikely climate variability was the cause. No climate variable affecting radial growth exhibited any significant long-term trends or differences between the early and late periods. Thus, we conclude that the age-specific changes in growth rates were unrelated to variations in climatic conditions within the region as measured via monthly values of temperature, precipitation, and drought severity, although we cannot exclude possible fine-scale temporal changes not recorded in monthly data. Likewise, the age-specific growth increases cannot be ascribed to the older trees becoming more canopy-dominant. There has been an overall trend towards woodland canopy closure in the Northern Rockies because of fire suppression activities (Keane *et al*., 2002). To avoid this potential confounding factor, we specifically selected trees in open, park-like environments where the impacts on radial growth of an individual tree are less likely to be influenced by competition from neighboring trees (Fig. 2). Further, this influence, if operative, would have existed before 1955–2004, and the age-specific differences in growth are evident only during the latter half of the 20th century. That is, radial growth responses are not age-specific ($P > 0.05$) during any other 50-year period dating to the mid-16th century. Finally, it is unlikely that these changes can be ascribed to nitrogen fertilization, as our sampled populations are from sites that have received minimal anthropogenic nitrogen deposition (Fenn *et al*., 2003).

One of the potential physiological causes for age-related growth declines in trees is associated with water limitations caused by increasing height (Sala & Hoch, 2009). Woodruff *et al*. (2004) discuss restrictions to growth related to tallness (and thus, indirectly, age) via gravitationally imposed constraints on turgor, and Ryan *et al*. (2006) show there are potential hydraulic constrictions associated with increasing height. In both cases, photosynthetic rates of taller trees are restricted either through the reduction of leaf and branch growth or through mid-afternoon stomatal closures as a means to reduce transpirational losses and thus avoid damage caused by cavitation. We found no significant relationships between tree height and diameter with radial growth responses at four of the sites and all sites combined. Thus, our results suggest that neither morphological characteristic is a marker for accelerated growth nor explains the increased growth in 1955–2004 relative to 1905–1954.

Ponderosa pine trees are highly vulnerable to water stress-induced xylem cavitation (Martínez-Vilalta *et al*., 2004) and compensate for this through increased stomatal control of transpirational losses (Piñol & Sala, 2000) and increased specific conductivity with age (Domec & Gartner, 2003). If xylem cavitation susceptibility increases with age in other conifers as shown by Domec & Gartner (2001), and suggested by Matthes *et al*., 2002,then the relative benefits of increased iWUE caused by increasing atmospheric CO_2 conditions may have preferentially favored the older trees and may explain the significant association between iWUE and radial growth (Fig. 8); a scenario we posit as most likely in this study. These results suggest that for ponderosa pine, height/cavitation relationships may actually be age/cavitation relationships that are often, but not always, associated with height. Older and taller trees have had more years to experience the cumulative effects of traumatic events (e.g., droughts) that have made them more susceptible to xylem cavitation;

subsequently, older trees may be more responsive to increases in iWUE.

Radial growth responses of OT and YT during 1850–2004 were marked by two distinct phases. From 1850 to 1935, OT and YT growth patterns were similar (Fig. 8) and no differences ($P > 0.05$) in averaged radial growth occurred. From 1940 onwards, OT always had higher radial growth indices than YT and the largest differences occurred during the most favorable growth periods. The initial pulse of growth in 1940 may have been related to a faster recovery of OT after the 1930s drought, but this pattern of above-average growth continued through the 2000–2004 pentad despite generally less favorable growing season conditions during the past two decades (e.g., Knapp & Soulé, 2007). OT maintained radial growth values above the mean and without a large decline since the peak growth period of the 1980s, which may indicate that a 'sustained enhancement' effect (cf. Kimball *et al*., 2007, p. 2181) is operative. A similar, but more muted growth response occurred for the younger trees.

In conclusion, these results demonstrate that old-growth ponderosa pine forests of the northern Rockies have likely benefited from the effects of increased atmospheric CO_2 since the mid-20th century and that the benefits increase with tree age. Further, the pronounced difference in radial growth indices beginning near mid-century suggests that a 'crossing of a CO_2 threshold' is required before discernible effects occur (Koutavas, 2008, p. 13). Radial growth increases in OT were significantly associated with rising iWUE, suggesting that accelerated growth rates are likely caused by more efficient water use in the semiarid environment where the trees were sampled. Because growth responses were largely consistent among the five study sites, the effects of increased iWUE appear operative on a regional scale.

Our findings also show that old-growth trees can be highly responsive to environmental changes and are capable of *increased* growth rates several 100 years after establishment (e.g., McDowell *et al*., 2003; Martínez-Vilalta *et al*., 2007) even in the absence of direct anthropogenic influences. The assumption of declining growth with age may not be fully operative, and increased productivity of old-growth forests documented by others may be in part because rising atmospheric CO_2 can alter growth responses. While we did not detect any significant changes in the climatic parameters affecting growth, the novel atmospheric CO_2 conditions since the mid-20th century were associated with increased iWUE that in turn preferentially favored older trees. Thus, there appears to be a significant capacity of old-growth ponderosa pine trees to respond to environmental change in the northern Rockies since the mid-20th century.

Acknowledgements

This project was funded by a USDA NRI Competitive Grant Program award–Plant Adaptations to the Environment #2005-35100-15226, and Faculty Research Grants from both the University of North Carolina at Greensboro and Appalachian State University. Samples were prepared for isotopic analysis at the Laboratory of Tree-Ring Research, and analyzed at the Environmental Isotope Laboratory of the Department of Geosciences, at the University of Arizona. We thank Steve Leavitt for his assistance with the isotopic component of the project, Steve Shelly, USDA Forest Service Region 1 RNA Coordinator, for identifying field sites and field assistance, and three anonymous reviewers for their constructive comments. Additionally, we thank Justin Maxwell, Jason Ortegren, Ian Snider, William Tyminski, and Philip White for field and/or laboratory assistance.

References

Allison CE, Francey RJ, Krummel PB (2003) $\delta^{13}C$ in CO_2 from sites in the CSIRO Atmospheric Research GASLAB air sampling network, (April 2003 version). In: *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, TN, USA.

Arneth A, Lloyd J, Santruckova H *et al*. (2002) Response of central Siberian Scots pine to soil water deficit and long-term trends in atmospheric CO_2 concentration. *Global Biogeochemical Cycles*, **16**, 1005, doi: 10.1029/2000GB001374.

Bert D, Leavitt SW, Dupouey J-L (1997) Variations of wood $\delta^{13}C$ and water-use efficiency of *Abies alba* during the last century. *Ecology*, **78**, 1588–1596.

Carey EV, Sala A, Keane R, Callaway RW (2001) Are old forests underestimated as global carbon sinks? *Global Change Biology*, **7**, 339–344.

Carrer M, Urbinati C (2004) Age-dependent tree-ring growth responses to climate in *Larix decidua* and *Pinus cembra*. *Ecology*, **85**, 730–740.

Cook ER (1985) *A time series analysis approach to tree-ring standardization*. PhD Thesis, University of Arizona, Tucson, AZ, USA.

Cook ER, Briffa K (1990) Comparison of some tree-ring standardization methods. In: *Methods of Dendrochronology* (eds Cook ER, Kairiukstis LA), pp. 153–162. Kluwer Academic Publishers, Dordrecht.

Cook ER, Peters K (1997) Calculating unbiased tree-ring indices for the study of climatic and environmental change. *The Holocene*, **7**, 361–370.

Coplen TB (1996) New guidelines for reporting stable hydrogen, carbon and oxygen isotope-ratio data. *Geochimica et Cosmochim ica Acta*, **602**, 3359–3360.

Domec J-C, Gartner BL (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. *Trees*, **15**, 204–214.

Domec J-C, Gartner BL (2003) Relationship between growth rates and xylem hydraulic characteristics in young, mature and old-growth ponderosa pine trees. *Plant, Cell and Environment*, **26**, 471–483.

Ehleringer JR, Cerling TE (1995) Atmospheric CO_2 and the ratio of intercellular to ambient CO_2 concentrations in plants. *Tree Physiology*, **15**, 105–111.

Etheridge DM, Steele LP, Langenfelds RL, Francey RJ, Barnola J-M, Morgan VI (1998) Historical CO_2 records from the Law Dome DE08, DE08-2, and DSS ice cores. In: *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, TN, USA.

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, **9**, 121–137.

Feng X (1999) Trends in intrinsic water-use efficiency of natural trees for the past 100–200 years: a response to atmospheric concentration. *Geochimica et Cosmochimica Acta*, **63**, 1891–1903.

Fenn ME, Baron JS, Allen EB *et al*. (2003) Ecological effects of nitrogen deposition in the western United States. *BioScience*, **53**, 404–420.

Francey RJ, Allison CE, Etheridge DM *et al*. (1999) A 1000-year precision record of $\delta^{13}C$ in atmospheric CO_2. *Tellus*, **51B**, 170–193.

Freyer HD (1979) On the ^{13}C record in tree rings. Part 1. ^{13}C variations in northern hemispheric trees during the last 150 years. *Tellus*, **31**, 124–137.

Grissino-Mayer HD (1996) A 2129-year reconstruction of precipitation for northwestern New Mexico, USA. In: *Tree-Rings Environment and Humanity* (eds Dean

JS, Meko DM, Swetnam TW), pp. 191–204. Department of Geosciences, University of Arizona, Tucson, AZ, USA.

Hemming DL, Switsur VR, Waterhouse JS, Heaton THE, Carter AHC (1998) Climate variation and the stable carbon isotope composition of tree ring cellulose: an intercomparison of *Quercus robur*, *Fagus sylvatica* and *Pinus silvestris*. *Tellus B*, **50**, 25–33.

Holmes RL (1983) Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin*, **43**, 69–78.

Huang J-G, Bergeron Y, Denneler B, Berninger F, Tardif J (2007) Response of forest trees to increased atmospheric CO_2. *Critical Reviews in Plant Sciences*, **26**, 265–283.

Hollander M, Wolfe D (1999) *Nonparametric Statistical Methods*, 2nd edn. John Wiley & Sons, New York.

Idso KE, Idso SB (1994) Plant responses to atmospheric CO_2 enrichment in the face of environmental constraints: a review of the past 10 years' research. *Agricultural and Forest Meteorology*, **69**, 153–203.

Kaufmann MR (1996) To live fast or not: growth vigor and longevity of old-growth ponderosa pine and lodgepole pine trees. *Tree Physiology*, **16**, 139–144.

Keane RE, Ryan KC, Veblen TT, Allen CD, Logan J, Hawkes B (2002) *Cascading effects of fire exclusion in the Rocky Mountain ecosystems: a literature review*. General Technical Report. RMRSGTR-91. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. Fort Collins, CO, 24pp.

Keeling RF, Piper SC, Bollenbacher AF, Walker JS (2008) Atmospheric CO_2 records from sites in the SIO air sampling network. In: *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, TN, USA.

Kimball BA, Idso SB, Johnson S, Rillig MC (2007) Seventeen years of carbon dioxide enrichment of sour orange trees: final results. *Global Change Biology*, **13**, 2171–2183.

Knapp PA, Soulé PT (2007) Trends in midlatitude cyclone frequency and occurrence during fire season in the Northern Rockies: 1900–2004. *Geophysical Research Letters*, **34** L20707, doi: 10.1029/2007GL031216.

Knapp PA, Soulé PT, Grissino-Mayer HD (2001) Detecting the potential regional effects of increased atmospheric CO_2 on growth rates of western juniper. *Global Change Biology*, **7**, 903–917.

Koutavas A (2008) Late 20th century growth acceleration in Greek firs (*Abies cephalonica*) from Cephalonia Island, Greece: a CO_2 fertilization effect? *Dendrochronologia*, **26**, 13–19.

LaMarche VC, Graybill DA, Fritts HC, Rose MR (1984) Increasing atmospheric carbon dioxide: tree-ring evidence for growth enhancement in natural vegetation. *Science*, **225**, 1019–1021.

Law BE, Thornton PE, Irvine J, Anthoni PM, Van Tuyl S (2001) Carbon storage and fluxes in ponderosa pine forests at different developmental stages. *Global Change Biology*, **7**, 755–777.

Leavitt SW, Danzer SR (1993) Method for batch processing small wood samples to holocellulose for stable-carbon isotope analysis. *Analytical Chemistry*, **65**, 87–89.

Leavitt SW, Idso SB, Kimball BA, Burns JM, Sinha A, Stott L (2003) The effect of long-term atmospheric CO_2 enrichment on the intrinsic water-use efficiency of sour orange trees. *Chemosphere*, **30**, 217–222.

Liu X, Shao X, Liang E, Zhao L, Chen T, Qin D, Ren J (2007) Species dependent responses of juniper and spruce to increasing CO_2 concentration and to climate in semi-arid and arid areas of northwestern China. *Plant Ecology*, **193**, 195–209.

Luo H, Oechel WC, Hastings SJ, Zulueta R, Qian Y, Kwon H (2007) Mature semiarid chaparral ecosystems can be a significant sink for atmospheric carbon dioxide. *Global Change Biology*, **13**, 386–396.

Luyssaert S, Schhulze E-D, Börner A *et al*. (2009) Old-growth forests and carbon sinks. *Nature*, **455**, 213–215.

Marshall JD, Monserud RA (1996) Homeostatic gas-exchange parameters inferred from 13C/12C in tree rings of conifers. *Oecologia*, **105**, 13–21.

Martínez-Vilalta J, Sala A, Piñol J (2004) The hydraulic architecture of Pinaceae – a review. *Plant Ecology*, **171**, 3–13.

Martínez-Vilalta J, Vanderklein D, Mencuccini M (2007) Tree height and age-related decline in growth in Scots pine (*Pinus sylvestris* L.). *Oecologia*, **150**, 529–544.

Matthes U, Kelly PE, Ryan CE, Larson DW (2002) The formation and possible ecological function of stem-strips in *Thuja occidentalis*. *International Journal of Plant Sciences*, **163**, 949–958.

McCarroll D, Loader NJ (2004) Stable isotopes in tree rings. *Quaternary Science Reviews*, **23**, 771–801.

McDowell N, Brooks JR, Fitzgerald SA, Bond BJ (2003) Carbon isotope discrimination and growth response of old *Pinus ponderosa* trees to stand density reductions. *Plant, Cell and Environment*, **26**, 631–644.

NCDC (2008) U.S. National/State/Divisional Data. Available at: http://www7.ncdc.noaa.gov/CDO/CDODivisionalSelect.jsp (accessed March 2009).

Palmer W (1965) *Meteorological Drought*. US Government Printing Office, Washington, DC, USA.

Phillips NG, Buckley TN, Tissue DT (2008) Capacity of old trees to response to environmental change. *Journal of Integrative Plant Biology*, **50**, 1355–1364.

Phipps RL (1985) *Collecting, preparing, crossdating, and measuring tree increment cores*. U.S. Geological Survey Water-Resources Investigations Report, pp. 85–4148.

Piñol J, Sala A (2000) Ecological implications of xylem cavitation for several Pinaceae in the Pacific Northern USA. *Functional Ecology*, **14**, 538–545.

Poorter H, Perez-Soba M (2001) The growth responses of plants to elevated CO_2 under non-optimal environmental conditions. *Oecologia*, **129**, 1–20.

Richardson DM, Rundel PW, Jackson ST *et al*. (2007) Human impacts in pine forests: past, present, and future. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 275–297.

Ryan MG, Phillips N, Bond BJ (2006) The hydraulic limitation hypothesis revisited. *Plant, Cell and Environment*, **29**, 367–381.

Ryan MG, Yoder BJ (1997) Hydraulic limits to tree height and tree growth. *BioScience*, **47**, 235–242.

Sala A, Hoch G (2009) Height-related growth declines in ponderosa pine are not due to carbon limitation. *Plant, Cell and Environment*, **32**, 22–30.

Saurer MS, Siegwolf RTW, Schweingruber F (2004) Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Global Change Biology*, **10**, 2109–2120.

Seibt U, Rajabi A, Griffiths H, Berry JA (2008) Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia*, **155**, 441–454.

Soulé PT, Knapp PA (2006) Radial growth rate increases in naturally-occurring ponderosa pine trees: a late 20th century CO_2 fertilization effect? *New Phytologist*, **171**, 379–390.

Stanton S (2007) Effects of dwarf mistletoe on climate response of mature ponderosa pine trees. *Tree Ring Research*, **63**, 69–80.

Sternberg LSL (1989) Oxygen and hydrogen isotope measurements in plant cellulose. In: *Modern Methods in Plant Analysis* (eds Linskens HF, Jackson JF), pp. 89–98. Springer-Verlag, Berlin.

Stokes MA, Smiley TL (1968) *Introduction to Tree-Ring Dating*. University of Chicago Press, Chicago.

Tang K, Feng X, Funkhouser G (1999) The $\delta^{13}C$ of trees in full-bark and strip-bark bristlecone pine trees in the White Mountains of California. *Global Change Biology*, **5**, 33–40.

Tognetti R, Longobucco A, Miglietta F, Raschi A (1998) Transpiration and stomatal behaviour of *Quercus ilex* plants during the summer in a Mediterranean carbon dioxide spring. *Plant, Cell and Environment*, **21**, 613–622.

Voelker SL, Muzika R, Guyette RP, Stambaugh MC (2006) Evidence for historic CO_2 enhancement of tree-ring growth shows a decline through age in *Quercus velutina, Quercus coccinea* and *Pinus echinata*. *Ecological Monographs*, **76**, 549–564.

Wang GG, Chhin S, Baurle WL (2006) Effect of natural atmospheric CO_2 fertilization suggested by open-grown white spruce in a dry environment. *Global Change Biology*, **12**, 601–610.

Wang X, Zhang Y, McRae DJ (2009) Spatial and age-dependent tree-ring growth responses of *Larix gmelinii* to climate in northeastern China. *Trees*, **23**, 875–885.

Waterhouse JS, Switsur VR, Barker AC, Carter AHC, Hemming DL, Loader NJ, Robertson I (2004) Northern European trees show a progressively diminishing response to increasing atmospheric carbon dioxide concentrations. *Quaternary Science Reviews*, **23**, 803–810.

Wigley TML, Briffa KR, Jones PD (1984) On the average value of correlated time series with applications in dendroclimatology and micrometeorology. *Journal of Climate and Applied Meteorology*, **23**, 201–213.

Williams JW, Jackson ST (2007) Novel climates, no analog communities and ecological surprises. *Frontiers in Ecology and the Environment*, **5**, 475–482.

Woodruff DR, Bond BJ, Meinzer FC (2004) Does turgor limit growth in tall trees? *Plant, Cell and Environment*, **27**, 229–236.

Wullschleger SD, Tschaplinski TJ, Norby RJ (2002) Plant water relations at elevated CO_2 – implications for water limited environments. *Plant, Cell and Environment*, **25**, 319–331.

Yamaguchi DK (1991) A simple method for cross-dating increment cores from living trees. *Canadian Journal of Forest Research*, **21**, 414–416.

Yoder BJ, Ryan MG, Waring RH, Schoettle AW, Kaufmann MR (1994) Evidence of reduced photosynthetic rates in old trees. *Forest Science*, **40**, 513–527.

Yu G, Liu Y, Wang X, Ma K (2008) Age-dependent tree-ring growth responses to climate in Qilian juniper *(Sabina przewalskii* Kom.). *Trees*, **22**, 197–204.

Zhou G, Liu S, Li Z *et al*. (2006) Old-growth forests can accumulate carbon in soils. *Science*, **314**, 1417.